

On the hardness of pigmented and unpigmented enamel in teeth of shrews of the genera *Sorex* and *Crocidura* (Mammalia, Soricidae)

By ELISABETH SÖDERLUND, E. DANNEID and D. J. ROWCLIFFE

*Department of Physical Metallurgy and Ceramics, Royal Institute of Technology, and Department of
Zoology, Stockholm University, Stockholm, Sweden*

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The hardness of the enamel in the incisors of three species of shrew, *Sorex araneus*, *S. minutus* and *Crocidura russula*, was tested using a special ultra-low load indentation instrument. Two different measuring areas were selected; the first area was situated in the anterior part of the incisor (covered with pigmented enamel in the *Sorex* spp.), the second area was in the posterior unpigmented part of the same tooth. Large local variations in the mechanical properties occur, and possible reasons for these are discussed. The results do not clearly confirm any hypothesis concerning differences in hardness between the pigmented and unpigmented enamel, although there is some tendency showing the unpigmented enamel to be slightly harder than the pigmented.

Introduction

The teeth of most shrews in the subfamily Soricinae are partly covered with reddish tooth pigment. The reddish colouring is most prominent on the labial part of the teeth and on the occlusal part of the molars. This colouring is due to the presence of iron (DÖTSCH and VON KOENIGSWALD 1978). The iron is confined to the outermost zone of the enamel. Pigmented enamel is also known in rodents (MILES 1963) and also in this case iron is present (SELVIG and HALSE 1975). Tooth pigment was present already in some fossil shrews from the Miocene (CROCHET 1975), but in this case we deal with members of another subfamily (REUMER 1987). It has been postulated that the iron-containing enamel should be harder than the white enamel (SELVIG and HALSE 1975) and thus function as a protection against abrasion (VOGEL 1984; DANNEID 1989). However, DÖTSCH (1982, pers. comm.) suggests that the iron-containing enamel is weaker than the unpigmented parts. The pigmented enamel is also said to be more acid resistant than the white enamel (SELVIG and HALSE 1975). Acid resistance was, however, outside the scope of this paper.

To the present authors' knowledge no direct studies of the mechanical properties, such as hardness, have been performed. This is certainly due to the difficulties of making adequate measurements in small volumes of material. However, a new type of sub-micron indentation system has recently been developed (PETHICA et al. 1983), built on the principle of a continuous and very precise reading of load and displacement. This system is particularly well suited for investigations of small specimens, such as the enamel of shrew teeth. In this study we have measured the hardness of both types of enamel on shrew teeth to see whether any distinction in hardness is apparent.

Material and methods

The hardness of the incisors of the lower left jaws of three different species of shrews, *Sorex araneus*, *S. minutus* and *Crocidura russula*, was measured. The two former species have the teeth partly covered with reddish enamel containing iron. *C. russula* has a very low content of iron in the enamel (VOGEL, pers. comm.) and was chosen for comparison.

The jaws were from museum specimens and therefore needed a careful treatment. They were mounted on a specimen holder by water-soluble glue and then indented as-received, even though a conventional specimen preparation with embedding and polishing of a cross-section would have been preferred. Three different specimens of each species were tested. The measurements were performed at two different positions of each incisor (Fig. 1); on an anterior part of the incisor, in the iron-

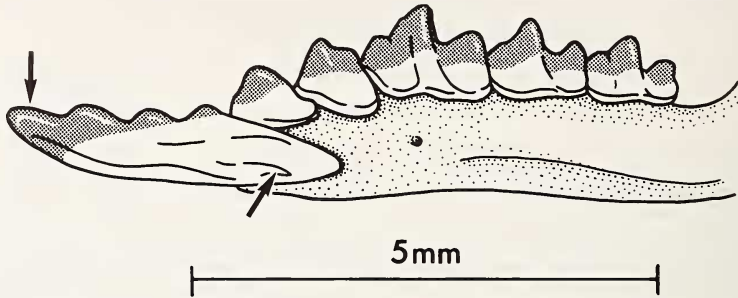


Fig. 1. Part of anterior left lower jaw of *S. araneus* with the measured areas indicated

containing area in the *Sorex* spp. or the corresponding area in *C. russula* (A), as well as in a more posterior position (B). At each position of the specimens several indentations were done, either in a line or in an ordered array.

The tests were performed with a special depth-sensitive indentation instrument (Nano indenter, Nano Systems Inc., TN, USA). The construction of the equipment and methods of analysis are described in detail elsewhere (PETHICA et al. 1983; DOERNER and NIX 1986). A schematic diagram of the indentation system is shown in Figure 2. The load is provided by a magnet and coil assembly and the corresponding displacement is continuously measured by the movement of the middle plate of a three-plate capacitance system. The depth-sensing mechanism makes optical imaging of the indentation unnecessary for the determination of the hardness, and in addition information from both the loading and the unloading sections of the experiment is recorded. The system is computer controlled and each indentation can be performed in an exact way by setting the approach rate, loading and unloading rate, time of hold and maximum load or displacement. The load and displacement resolutions are 0.3 μN and 0.2 nm, respectively.

In this study indentations were made with maximum displacements of 100 and 250 nm, i.e. the maximum depth of indentation is only a fraction of the expected thickness of the pigmented part of the enamel of 40–50 μm (MILES 1963; VOGEL 1984). The loading and unloading rates were 10 nm/s. A 30 s hold segment (a segment during which the load is held constant for a certain period of time) was applied at maximum load and at 90% of unloading to check the thermal drift. The result from a single indentation experiment is typically presented as a load-displacement curve (Fig. 3). The hardness is calculated as the maximum load divided by the area of the indentation after subtraction of elastic contributions.

The microstructure was studied and a qualitative elemental analysis of the enamel was done using a scanning electron microscope (SEM – JSM-840, JEOL Ltd, Tokyo, Japan) equipped with an energy dispersive spectrometer (EDS – LINK AN 10 000, Link Analytical Systems Ltd, High Wycombe, UK).

Results

The nanoindentation system is very sensitive to differences in height across the specimen, as well as to unevennesses in the specimen surface. Nevertheless, the method of specimen mounting worked satisfactorily as long as the indentations were placed on nearly horizontal areas close to the highest point of the samples. The surfaces of the teeth at the microlevel

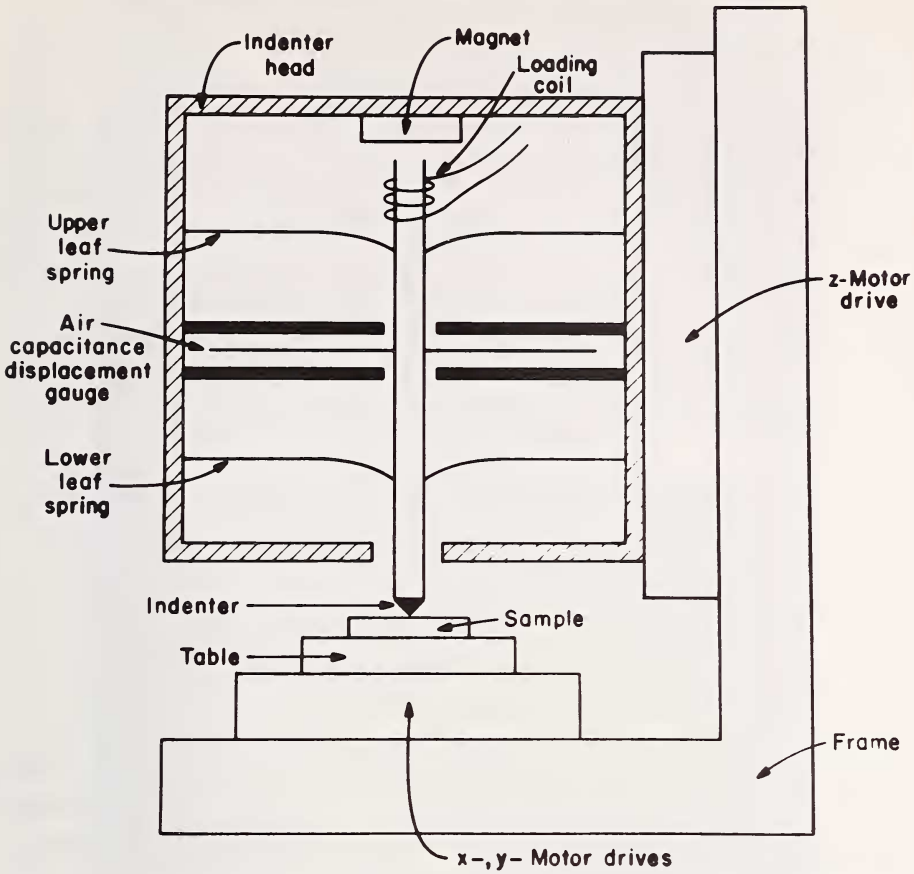


Fig. 2. Schematic diagram of the indentation system (from SÖDERLUND and MACMILLAN 1991)

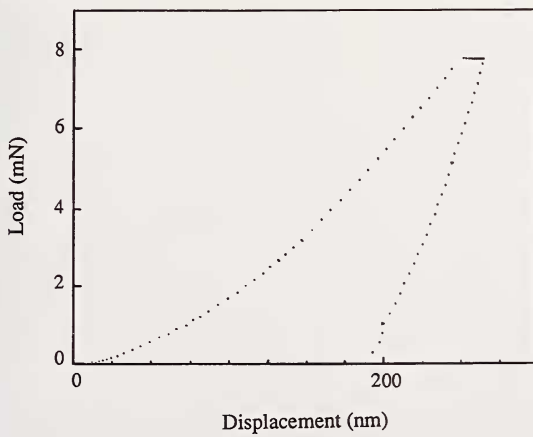


Fig. 3. Typical load-displacement curve obtained from a 250 nm indentation in the unpigmented enamel of a *S. araneus* specimen

are very rough (Fig. 4), but the load-displacement curves reveal any tendency of sliding of the indenter or other abnormality and such indentations were then disregarded. The thermal drift in the system was measured and was typically less than 0.05 nm/s.

Most of the hardness data lay in the regime 6–9 GPa (hereafter called the high regime) and in some cases around 0.1–0.4 GPa (henceforth named the low regime) for a 100 nm deep impression. Values obtained from 250 nm deep indentations were of the order 4–7 GPa or lay in the same low regime of 0.1–0.4 GPa. The hardness data obtained from one of the *S. minutus* specimens lay only in the low regime. The data points in Figure 5 are the

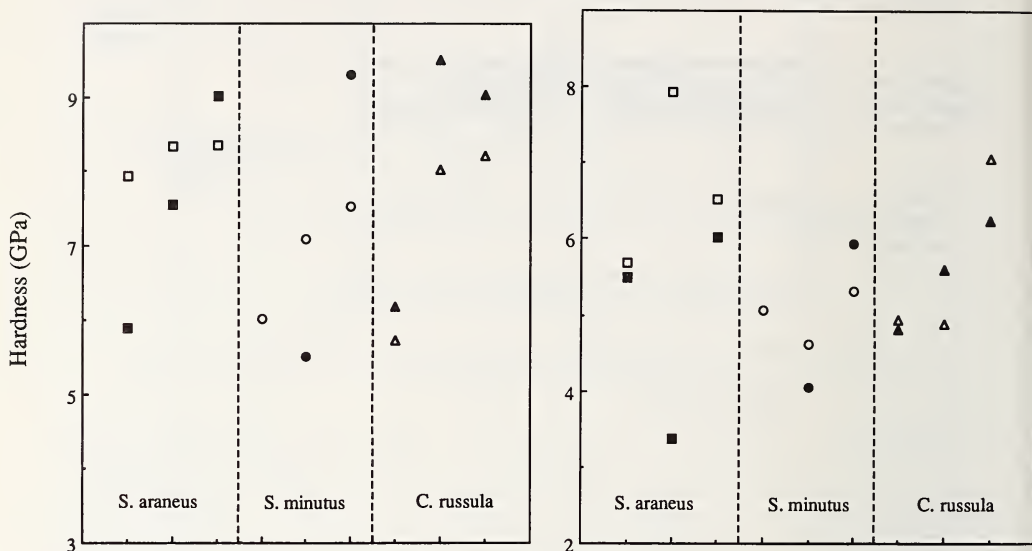
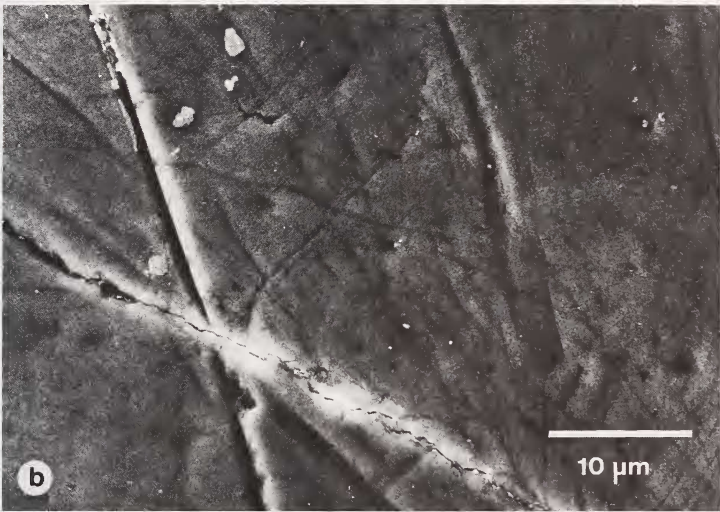


Fig. 5. The average hardness (high regime) obtained from area A of each specimen (filled symbols) and from area B (unfilled symbols) for the two indentation depths 100 nm (a) and 250 nm (b)

averages of the high regime values obtained at each position of each specimen. The Table presents the mean high regime values together with one standard deviation and the number of valid indentations. The percentage of the total number of indentations that lay in the low hardness regime for each region is seen in the Table. The values in the low regime could not be used for comparative purposes for several reasons, which are discussed in the next section.

The SEM study revealed that the surface of the enamel was rough (Fig. 4). Commonly, the enamel was very scratched and cracks could be seen. Occasionally the enamel had fractured and pieces of the enamel were missing. The *S. minutus* specimen with hardness values only in the low regime, seemed to be covered with a thin film. However, no difference in composition could be detected by EDS, between areas of enamel with or without the film. By the EDS analysis it was confined that the pigmented areas were rich in iron. In areas where the iron content was higher than the surroundings, the concentration of calcium was correspondingly lower. The oxygen concentration was enhanced in the same areas as the iron.

Fig. 4. SEM micrographs of a *S. araneus* specimen showing (a) the microstructure of the pigmented enamel (position A), (b) the microstructure of the unpigmented enamel (position B), and (c) two of the indentations made in position A



Average of hardness (high regime), standard deviation and number of indentations for the two regions tested at each specimen

Specimen and species	Measured area on tooth	Hardness (in GPa)	Standard deviation (in GPa)	Number of indentations	Indentations in the low regime (% of total number)
Indentation depth = 100 nm					
<i>S. araneus</i> 1	A	5.88	1.27	10	31.3
<i>S. araneus</i> 1	B	7.93	1.45	8	20.0
<i>S. araneus</i> 2	A	7.55	2.31	5	0.0
<i>S. araneus</i> 2	B	8.34	0.99	5	0.0
<i>S. araneus</i> 3	A	9.01	2.49	19	56.4
<i>S. araneus</i> 3	B	8.36	2.85	18	5.0
<i>S. minutus</i> 1	A	—	—	8	97.4
<i>S. minutus</i> 1	B	6.01	1.70	5	61.5
<i>S. minutus</i> 2	A	5.51	1.45	8	20.0
<i>S. minutus</i> 2	B	7.09	1.72	8	65.0
<i>S. minutus</i> 3	A	9.30	2.72	13	28.6
<i>S. minutus</i> 3	B	7.54	0.59	7	10.0
<i>C. russula</i> 1	A	6.18	1.89	9	55.0
<i>C. russula</i> 1	B	5.72	0.93	11	20.0
<i>C. russula</i> 2	A	9.51	3.56	10	0.0
<i>C. russula</i> 2	B	8.03	1.03	7	10.0
<i>C. russula</i> 3	A	9.04	3.65	8	30.0
<i>C. russula</i> 3	B	8.21	1.34	7	0.0
Indentation depth = 250 nm					
<i>S. araneus</i> 1	A	5.50	1.54	7	0.0
<i>S. araneus</i> 1	B	5.69	0.82	9	0.0
<i>S. araneus</i> 2	A	3.38	0.74	5	0.0
<i>S. araneus</i> 2	B	7.93	1.62	4	0.0
<i>S. araneus</i> 3	A	6.03	1.35	14	34.6
<i>S. araneus</i> 3	B	6.52	0.88	16	0.0
<i>S. minutus</i> 1	A	—	—	8	94.9
<i>S. minutus</i> 1	B	5.06	1.71	8	0.0
<i>S. minutus</i> 2	A	4.04	1.10	9	10.0
<i>S. minutus</i> 2	B	4.62	0.78	12	40.0
<i>S. minutus</i> 3	A	5.93	0.77	4	20.0
<i>S. minutus</i> 3	B	5.32	0.52	9	10.0
<i>C. russula</i> 1	A	4.82	0.93	13	35.0
<i>C. russula</i> 1	B	4.94	1.09	15	0.0
<i>C. russula</i> 2	A	5.59	2.11	10	0.0
<i>C. russula</i> 2	B	4.89	1.04	9	10.0
<i>C. russula</i> 3	A	6.24	1.18	10	0.0
<i>C. russula</i> 3	B	7.06	1.29	10	0.0

Discussion

The large scatter in the hardness data indicates a substantial inhomogeneity in the surface properties. In particular the occurrence of the low and high hardness regimes can only be interpreted as arising from different materials. We suggest that the high hardness regime reflects the hardness of more or less undamaged enamel, whereas the low hardness sites would occur if the enamel was covered with a thin, soft film or locally absent and the impressions were then being made in the underlying softer structure. For this reason the low regime values are not considered further in this study.

The different mean levels of the high regime hardness values corresponding to the two

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